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Multidrug Resistant Bacteria in Air Samples from Indoor Environment in Kathmandu, Nepal: A Cross-Sectional Study

Sanju Sah[1], Aruna Khanal[1]

ABSTRACT

Background: Indoor air flora has been linked to several allergic and infectious diseases; hence, monitoring of air quality is key to achieving better health outcomes. It is therefore imperative to monitor air quality for antibiotic-resistant bacteria that are on the rise and are associated with poor human health.

Methods: The study was conducted from June 2019 to November 2020 across 24 indoor sites in Kathmandu Valley, Nepal. Air samples were collected using an impactor air sampler and cultured on various media. Bacterial isolates were identified, and their antibiotic susceptibility patterns were determined using the Kirby-Bauer method per CLSI 2018 guidelines.

Results: A total of 73 culture plates representing 24 samples, were examined. The predominant bacteria included Staphylococcus spp. and Citrobacter freundii. Among 50 isolates, 72% were multidrug- resistant. Gentamycin and Ofloxacin were the most effective antibiotics. Hospitals exhibited the highest microbial load (2729 CFU/m^3).

Conclusion: The presence of multidrug-resistant bacteria in indoor environments of Kathmandu highlights a significant public health concern. Continuous monitoring is essential for preventive action.

Keywords: Bacterial contamination, indoor air pollution, antimicrobial resistance

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[1]Department of Microbiology, St. Xavier's College, Kathmandu, Nepal

Correspondence to:
Sanju Sah
Department of Microbiology,
St. Xavier's College, Kathmandu,
Nepal
Email: sahsanju57@gmail.com

BACKGROUND

Indoor air is an important aspect of the human sphere of life since the majority of the population spent substantial amounts of time within the precincts of buildings. The most common sources of microorganisms in the air are soil, microorganisms found in water, plant or animal surfaces, and human beings (by activities like coughing, sneezing, talking, and laughing).(1) Various factors affect the presence of a microorganism in the air, such as atmospheric temperature, humidity, air current, etc.(2) An important area of concern for human public health is the incidence of antibiotic resistance among pathogenic and opportunistic bacteria of different sources, and this emerging issue not only represents a nosocomial problem but also involves a variety of environments.(3) This study was carried out to assess the pathogenic and opportunistic bacteria along with the multi-drug-resistant (MDR) bacteria in air samples from indoor environments of Kathmandu valley. Multidrug resistance is defined as the acquired resistance to at least one agent from three or more antimicrobial categories.(4) Many bacterial infections, halted by a wide range of antibiotics, are responsible for causing mortality and morbidity due to the evolution and procurement of antibiotic-resistant genes.(5) Air pollution is one of the major public health concerns in developing countries, as a majority of the population of Kathmandu Valley in Nepal is in threat of acquiring disease due to air pollution.(6) The organisms causing such diseases are becoming increasingly resistant to antibiotics. (7) Therefore, the timely monitoring of the air quality could allow the government to take corrective measures. This study will help to evaluate the microbial air quality of the indoor environment in terms of bacterial load.

METHODS

This descriptive cross-sectional study was conducted to assess multidrug-resistant (MDR) bacteria in air samples from indoor environments in the Kathmandu Valley, Nepal. Air samples were collected from six hospitals, six offices, six schools, and six colleges across various districts of the Kathmandu Valley, and were processed at the Department of Microbiology, St. Xavier's College. The study was carried out from June 2019 to November 2019. Samples were collected using an impactor air sampler, which collects 300 liters of air in 5 minutes. Indoor air samples were taken from different areas, including classrooms in schools and colleges, emergency wards in hospitals, and administrative rooms in offices.

The sample size was calculated using the formula: $n=\frac{Z^2 pq}{d^2}$

The prevalence of death due to respiratory diseases because of indoor air pollution has been reported as 1%.(8)

Sample size = (1.96) 2 x0.01x0.99/0.0025

= 14.5~15 (p, prevalence; d, allowable error)

Where the prevalence of respiratory diseases due to indoor air pollution was considered to be 1%. The calculated sample size was 15, but to increase statistical power and data reliability, a total of 24 samples were collected, with each sample processed in triplicate to enhance accuracy. The independent variables in the study included groups of people (children, adults, workers), activities (walking, working), and room height (e.g., floor level). The dependent variable was the presence of bacteria. The inclusion criteria for this study were the indoor air from hospitals, schools, colleges, and offices, as people spend significant time in these environments, affecting air quality and health. The exclusion criteria were open environments, as the air sampler was designed for indoor use, and sites lacking controlled indoor areas, poor ventilation, or minimal human activity that could affect air quality. An ANOVA test was used to determine the p-value, as it allows for the comparison of means across multiple groups to assess statistical significance. Data were analyzed using SPSS version 19.

Samples were collected from the sites using an impactor air sampler on agar plates, then transported to the laboratory within 1–2 hours and incubated at 37°C for 24 hours. After incubation, colony counts and characteristics were analyzed, followed by microscopic examination using Gram staining and biochemical testing on nutrient agar. The colony-forming units (CFUs) of predominant bacteria were enumerated from three Blood Agar plates, three MacConkey Agar plates, one Cetrimide Agar plate, and one Mannitol Salt Agar plate per sampling site. CFUs were used to determine microbial load. The microbial load was calculated using the formula: Cfu/m3=No. of colonies observed ×1000/ volume of the air sample. Antimicrobial susceptibility was tested using the Kirby-Bauer disc diffusion method, and multidrug-resistant organisms were identified according to CLSI 2018 guidelines. Antibiotics tested for Gram-positive bacteria included penicillin, cefoxitin, erythromycin, vancomycin, gentamicin, and ciprofloxacin. These agents target the bacterial cell wall or protein synthesis.(9) For Gram-negative bacteria, ampicillin, third-generation cephalosporins, gentamicin, and ciprofloxacin were used. Antibiotics commonly used against

Ethical approval for this study was obtained from Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal. Written permission was also obtained from the sampling sites.

RESULTS

A total of 73 culture plates representing 24 samples, were examined. These samples were collected from six locations each: hospitals, schools, colleges, and offices within the Kathmandu Valley, where 14 (28%) bacteria from hospitals, 13 (26%) from offices and schools, and 10 (20%) from colleges were isolated and there was no bacterial growth in other 103 culture plates (Figure 1).

This study shows that hospitals had the highest levels of airborne bacteria, with 14 (28%) isolates, followed by offices and schools with 13 (26%), and colleges with 10 (20%).

Based on Sanitary Standards for Non-industrial premises formulated by the European Commission, the schools were found to have a high level of air pollution, ranging from 500 to 2000 CFU/m^3, while hospitals, colleges, and offices exhibited very high levels, exceeding 2000 CFU/m^3. Degree of air pollution was significantly associated with the mean concentration of bacterial loads of different sampling sites (p=0.05) (Table 1).

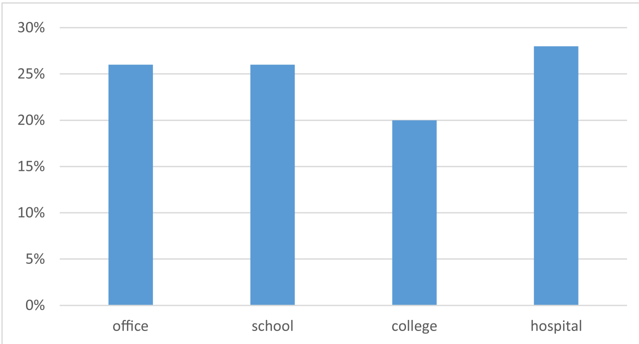

Figure 1: Growth profile of bacteria in air samples

Table 1. An Assessment of air quality in the selected sites according to the Sanitary Standards from Non-industrial Premises

Sampling Sites	Mean no. of Colonies (Observed)	Mean no. of Colonies Calculated (Cfu/m)	Air Pollution (Degree)	p-value
Hospital	726.6	2728.7	Very high	
College	615.9	2234.4	Very high	0.05
School	474.05	1318.45	High	
Office	661.1	2041.35	Very high	

Based on Sanitary Standards for Non-industrial premises formulated by the European Commission, the schools were found to have a high level of air pollution, ranging from 500 to 2000 CFU/m^3, while hospitals, colleges, and offices exhibited very high levels, exceeding 2000 CFU/m^3. The degree of air pollution was found to be significantly associated with the mean concentration of bacterial loads across different sampling sites (p = 0.05), as determined by ANOVA test (Table 1).

When a total of 33 Staphylococcus aureus isolates were tested for antimicrobial susceptibility, Gentamycin (93.9%) was found to be the most effective drug, followed by Ofloxacin (90.9%), respectively. Staphylococcus aureus 2575.7%) were resistant to cefoxitin and hence were considered to be Methicillin-resistant Staphylococcus aureus (Table 2).

When a total of 11 Staphylococcus epidermidis isolates were tested for antimicrobial susceptibility, Ofloxacin (100%) was found to be the most effective drug, followed by Gentamicin (90.9%), Cefazolin (90.9%), and Novobiocin (90.9%), respectively (Table 3).

Eleven Staphylococcus saprophyticus isolates were tested for antimicrobial susceptibility, out of which Ofloxacin, Gentamycin, Amikacin, Cefazolin, and Chloroamphenicol were found to be the most effective drugs (100%), followed by Cefoxitin and Azithromycin, which showed 60% sensitivity (Table4). Among 49 Gram-positive bacterial isolates obtained from air samples, the prevalence of multidrug resistance was seen at 24.2% in Staphylococcus aureus and 9% in Staphylococcus epidermidis. Staphylococcus aureus isolated from hospital (H2) was found to be resistant to five antibiotics belonging to different classes (Azithromycin, Ceftazidime, Ofloxacin, Cephalixin, and Cefazolin). Similarly, in the case of Staphylococus epidermidis isolated from school (S6), resistance was found towards four antibiotics of different classes (Azithromycin, Ceftazidime, Amikacin, and Cefoxitin) (Table 5).

Table 2. Antibiotic sensitivity pattern of S. aureus

Antibiotic Class	Antibiotic (mcg)	Sensitive	Intermediate	Resistant
		No. (%)	No. (%)	No. (%)
β-lactum	Ceftazidime (30)	9(27.2)	10(30.3)	14(42.4)
	Cefoxitin (30)	8(24.2)	0(0)	25(75.7)
	Cefazolin (30)	27(81.8)	0	6(18.1)
Aminoglycoside	Amikacin (30)	19(57.5)	7(21.2)	7(21.2)
	Gentamicin (30)	31(93.9)	1(3.0)	1(3.0)
Macrolide	Azithromycin (15)	9(27.2)	0(0)	24(72.7)
Fluroquinolones	Ofloxacin (5)	30(90.9)	1(3.0)	2(6.0)
Chloramphenicol	Chloramphenicol (30)	28(84.8)	0(0)	5(15.2)

Table 3. Antibiotic sensitivity pattern for S. epidermidis

Antibiotic Class	Antibiotic (mcg)	Total S. epidermidis isolates (n= 11) Sensitive	Intermediate	Resistant
		No. (%)	No. (%)	No. (%)
β –lactum	Ceftazidime (30)	4(36.36)	2(18.1)	0(0)
	Cefoxitin (30)	7(63.6)	0(0)	4(36.3)
	Cefazolin (30)	10(90.9)	1(9.0)	0(0)
Aminoglycoside	Amikacin (30)	7(63.6)	2(18.1)	2(18.1)
	Gentamicin (30)	10(90.9)	0(0)	1(9.0)
Macrolide	Azithromycin (15)	7(63.6)	0(0)	4(36.3)
Fluroquinolones	Ofloxacin (5)	11(100)	0(0)	(0)
Chloramphenicol	Chlorampheni-col(30)	8(72.7)	1(9.0)	2(18.1)
Aminocoumari	Novobiocin(30)	10(90.9)	0(0)	0(0)

Table 4. Antibiotic sensitivity pattern of S. saprophyticus

Antibiotic Class	Antibiotic (mcg)	Total S. saprophyticus isolates(n=5) Sensitive	Intermediate	Resistant
		No. (%)	No. (%)	No. (%)
β -lactum	Ceftazidime (30)	2(40)	1(20)	2(40)
	Cefoxitin (30)	3(60)	0(0)	2(40)
	Cefazolin (30)	5(100)	0(0)	0(0)
Aminoglycoside	Amikacin (30)	5(100)	0(0)	0(0)
	Gentamicin (30)	5(100)	0(0)	0(0)
Macrolide	Azithromycin (15)	3(60)	2(40)	0(0)
Fluroquinolones	Ofloxacin (5)	5(100)	0(0)	0(0)
Chloramphenicol	Chloramphenicol (30)	5(100)	0(0)	0(0)
Aminocoumarin	Novobiocin(30)	0(0)	0(0)	5(100)

Table 5. MDR patterns of gram-positive bacteria

Isolates	No. of isolates showing resistance	Degree of resistance R3	R4	R5	Total MDR (≥ R3)
S. aureus (33)	25	6	1	1	8(24.2%)
S. epidermidis (11)	10		1		1(9 %)
S. saprophyticus (5)	5				
C. freundii (n= 1)	1				

Note: R3 indicates resistance to 3 antibiotics. R4 and R5 represent resistance to 4 and 5 antibiotics, respectively.

DISCUSSION

In this study, a total of 24 air samples, 6 each from different emergency wards of hospitals, administrator rooms of offices, and class rooms of colleges and schools, were selected randomly, was collected using an impactor air sampler. In this study, the number of gram- positive bacterial isolates was higher than gram-negative bacterial isolates. This may be due to the relative humidity that has been shown to be of major importance in the survival of microorganisms. In general, Gram-positive bacteria are more tolerant to desiccation, whereas Gram-negative bacteria react unfavourably to the desiccation because the amount of lipid bilayer is higher in them than that of gram-positive bacteria. This can be a possible reason for the higher prevalence of Gram-positive bacteria in indoor environments.(10)

Blood agar was used for total plate count as it is an enriched medium that supports growth for fastidious organisms. In this study, the air quality of emergency wards of hospitals was found to be intermediate, having an indoor air bacterial load between 100 and 500 Cfu/m^3. This might be due to high patient flow and density of health personnel and other individuals.(11) According to the Sanitary Standard for Non-Industrial Premises, European Commission, indoor air having a bacterial load between 100 and 500 cfu/m3 is considered to be intermediately polluted.(12) From the emergency wards of 6 hospitals, a total of 14 bacterial isolates were obtained. Among that, 8 (57%.1.) were Staphylococcus aureus, 5 (45.4%) were Staphylococcus epidermidis, and 1 (100%) was Citrobacter freundii. The most common source of Staphylococcus aureus is sneezing, coughing, and spitting of saliva from an infected person colonized with the organisms.(13) This study showed the presence of Staphylococcus species of bacteria more dominant in the ambient air of all the sample sites, followed by an extremely low number of Gram-negative bacteria. This may be due to the type of activities carried out in the study site, the type and number of patients at the site, the level of ventilation, and the level of hygiene of the study site.(14) In this study, the administrator rooms of all offices were found to have an intermediate indoor air bacterial load between 100 and 500 cfu/m3. This might be due to the large number of workers, visitors, and their activities, furnishing materials, and poor ventilation and air-conditioning systems of all offices.(15) Out of total 13 isolates found in hospitals, 5 (15.1%) were Staphylococcus aureus, 4 (36.4%) were Staphylococcus epidermidis, and 4 (80%) were Staphylococcus saprophyticus. In this study, while comparing the classrooms of all colleges and schools, each college was found to be very highly polluted, while the school was found to be highly polluted. Out of 6 colleges studied, a total of 10 (30.3%) bacterial isolates were obtained, i.e., all were Staphylococcus aureus. And a total of 13 isolates were obtained from schools, among those, 10 (30.3%) were Staphylococcus aureus, 2 (18.2%) were Staphylococcus epidermidis and 1 (20%) was Staphylococcus saprophyticus. This might be due to college and school staffs and density of students, inadequate ventilation, and higher movement of students during the time of sample collection.(16) Constant re-suspension of particles from room surfaces is also responsible for the presence of microorganisms in indoor air.(17) In this study, schools were found to have a high degree of air pollution between 500–2000 cfu/m^3, followed by colleges which exhibited very high levels of air pollution greater than 2000 cfu/m^3. In the similar study conducted, a mean indoor value of the bacterial concentration was found to be 5105 cfu/m^3 in an urban area, clearly exceeding the limit value of 500 for an indoor environment.(18) In this study, the most effective antibiotics against S. aureus were Gentamycin (93.9%), followed by Ofloxacin (90.9%). MDR Staphylococcus aureus showed the highest resistance toward cefoxitin (75.5%) and azithromycin (72.7%). Among the antibiotics used for S. epidermidis, Ofloxacin (100%), Gentamycin (90.9%), and Cefazolin (90.9%) showed the highest sensitivity. The antibiotic susceptibility pattern of S. saprophyticus revealed that 100% sensitivity was seen for Ofloxacin, Gentamicin, Amikacin, Cefazolin, and Chloramphenicol. This finding was similar to the study conducted by Kunwar et al. (2019), in which out of 16 indoor air samples, S. aureus (47.18%) and Pseudomonas spp. (1.82%) were isolated, Gram-positive organisms like CoNS, Streptococcus spp., Micrococcus spp., and Bacillus spp. and Gram- negative organisms like E.coli and Proteus spp. were identified. The most effective antibiotics against S. aureus were Gentamycin (81.81%) and Ofloxacin (81.81%). The P- value was found to be < 0.05 i.e. (0.040), which explains that there exists an association between the number of bacteria isolated from the classrooms of different schools and the emergency wards of different hospitals.

Out of 33 Staphylococcus aureus isolates, 8 were found to be sensitive to the antibiotics tested, while 25 isolates exhibited resistance, indicating the presence of multidrug-resistant (MDR) S. aureus strains in the indoor environment of Kathmandu Valley. However, limited data are available on MDR bacteria from indoor environments in this region, with most existing studies focused on hospitals. Research specifically investigating the indoor air of schools, colleges, offices, and hospitals in Nepal remains scarce. This study reports multidrug-resistant bacteria in the indoor environment of Kathmandu Valley. Therefore, there is a need for timely indoor air quality monitoring.(19) These findings widen the information on the current status of the multidrug-resistant bacteria in the indoor environment of Kathmandu Valley in Nepal. As part of the master's curriculum, the research was limited to 6 days a week, 5 hours per day, for 6 months in the college laboratory, restricting the number of samples. Therefore, only a limited number of samples were taken from indoor environments in Kathmandu Valley. Other areas within hospitals, schools, colleges, and offices were not sampled, and seasonal variations could not be explored due to the fixed sampling period.

CONCLUSION

The study shows prevalence of MDR strain of Staphylococcus spp. in indoor environment of different facilities (hospitals, offices, schools and colleges) of Kathmandu valley, which is a public health threat to the residents of Kathmandu. Gentamycin and Ofloxacin were the most effective antibiotics against such MDR strains and also for isolated Gram-positive bacteria.

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Conflict of Interest

The authors declare no conflict of interest.

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